ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

COMPLEMENT-FIXATION TESTS FOR ANTISERA PREPARED AGAINST STRAIN HELA CELL AND 5 CELL STRAINS DERIVED FROM HUMAN BREAST CARCINOMA

BY

EERO MUSTAKALLIO and J. A. GRÖNROOS

VOL. 35 1957 SUPPLEMENTUM 12

MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

DEPARTMENT OF MEDICAL MICROBIOLOGY, UNIVERSITY OF TURKU

COMPLEMENT-FIXATION TESTS FOR ANTISERA PREPARED AGAINST STRAIN HELA CELL AND 5 CELL STRAINS DERIVED FROM HUMAN BREAST CARCINOMA

BY

EERO MUSTAKALLIO and J. A. GRÖNROOS

TURKU . UUDEN AURAN OY:n KIRJAPAINO . 1957

INTRODUCTION*

Numerous immunological experiments have been carried out with normal and tumour tissues both in vivo and in vitro. The papers published up to 1952 in this field have been reviewed by Hauschka in an excellent article which reveals that the advance has yet been limited. Relatively few papers have, however, dealt with the immunological behaviour of cells of human origin maintained in tissue culture. Mountain described (5) the cytopathogenic effect of rabbit antisera prepared with HeLa cells derived from portio carcinoma (2) on HeLa cells in tissue culture. She found that these antisera were capable of agglutinating and lysing the HeLa cells in culture. In contrast, sera of rabbits immunized with the maintenance solution employed did not affect HeLa cells in culture. The anti-HeLa action could be removed by absorption with HeLa cells.

In 1956 Miller and Hsu (4) noted that rabbit and chicken anti-HeLa sera exhibited a cytotoxic activity against a variety of tissue cultures of human cells including fibroblasts and epithelium from human tonsil and skin, fibroblasts from fetal muscle and human leukocytes. The effect was thus not directed specifically against HeLa cells.

It may be of interest in this connection to mention Horn's report on the cytotoxicity of various antisera prepared against Ehrlich mouse ascites tumour cell components (3). Using ascites tumour cells as antigen the author observed that the antisera agglutinate HeLa cells and that complement fixation occurs. These reactions could also be seen in immune sera prepared using cell components.

The present report describes complement-fixation tests carried out with antisera prepared with HeLa cells and five cell strains derived from human breast carcinoma which are being maintained in tissue culture.

---

* This paper was presented at the Meeting of the Finnish Association of Pathologists at Helsinki, May 10th, 1957.

MATERIALS AND METHODS

Cell Strains. — The strain HeLa cell was originally from J. T. Syverton, M.D., Minneapolis, and was kindly supplied by Prof. Oker-Blom, Helsinki. Cultures were made of five primary human female breast carcinoma tissues by following mainly the technique described by Orr and Swain (6). Table 1 shows the date of isolation, diagnosis of the histologic section of the primary tumour and time the cell strains were maintained. The ages of these cell strains varied from 15.5 to 24 months. Two media were employed, one composed of Parker's maintenance solution 199 (48 %), human serum (50 %) and embryo extract (2 %), and the other of Eagle's solution (78 %), human serum (20 %) and embryo extract (2 %). Both the solutions were manufactured by Orion Oy, Helsinki. Penicillin and streptomycin were added to media in such amounts that the final concentration of each was 100 microg/ml serum. The nutrient solution was renewed routinely twice a week and culture transfers were made every other week according to the directions of Syverton and Scherer (7). Fig. 1 shows unstained living cultures of the strains investigated in the present study.

TABLE 1

STRAINS DERIVED FROM HUMAN BREAST CARCINOMA

Strain	Date of Isolation	Histologic Section of Primary Tumor*	Number of Months Maintained in Culture
EG	9. 5. 55	Invasive duct adenocarcinoma	24
EJ	23. 5. 55	Scirrhous solid (partly adenomatous) carcinoma	23.7
EV	15. 9. 55	Non differentiated epidermoid carcinoma	20
AR	22. 9. 55	Invasive duct adenocarcinoma	19.6
LR	26. 1. 56	Mainly solid carcinoma simplex	15.5

Preparation of Immune Sera. — The immunisations were carried out using rabbits close to one year old. For the preparation of antigenic material, the cells were liberated from the glass surface by means of trypsin, washed three times in Hank's solution (manufactured by Orion Oy), centrifuged at 1500 rpm for 5 minutes and then diluted 1:100.

* Examined by Prof. Osmo Järvi, M.D., Department of Pathology, University of Turku.

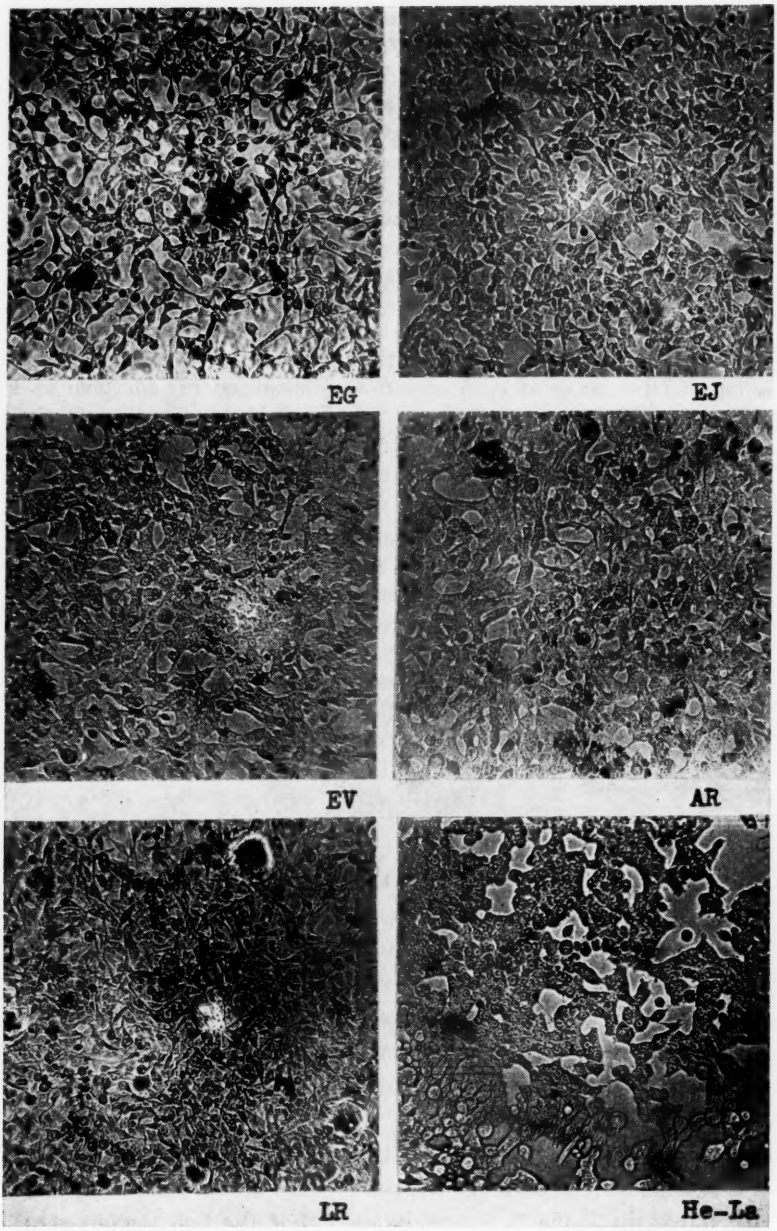

Figure 1. Unstained living cultures of the cell strains investigated. Strains EG, AR and LR were photographed three days, strain HeLa five days, and strain EJ and EV six days after the last transfer.

The suspension contained approximately 5×10^5 cells per ml. The freshly prepared antigen was injected intravenously in volumes of 0.25, 0.5 and 1.0 ml at intervals of five days. Six days after the last immunisation the animal was bled by severing the carotid artery.

Test Schedule. — Because no satisfactory endpoint of the agglutination reaction was observed, it was necessary to resort to complement-fixation tests (CFT). The antigen was prepared in the same way as for the immunisations. The concentrations of the antigen were checked. Although no complement-fixation occurred when undiluted antigen was employed, the dilution 1:100 was chosen for the immunisation because it gave a satisfactory complement-fixation and because the consumption of cells was relatively low. The CFT was carried out mainly according the directions given by Winkle (8). Antigen, antiserum and two units of complement were incubated for one hour in a 37° water bath. The haemolytic system was added and the tests were read after incubating 15 and 60 minutes at 37° C by taking 50 percent haemolysis as the endpoint.

Absorption Technique. — The relationships between the various antigens were determined by cross-absorption tests. These were carried out using cells treated in the same way as in the preparation of antigens with the exception that packed cells were used. Cells and immune sera diluted 1:10 were mixed in equal volumes and the mixtures incubated for one hour at 37° C, followed by 18 hours at +4° C. The mixture was then centrifuged and the supernatant absorbed serum removed for use in the complement-fixation tests.

RESULTS

The figures in Table 2 give the highest dilutions of antisera which produced 50 percent haemolysis. The dilutions were twofold beginning with the dilution 1:10. All reactions have been run a number of times. The range of variation was with one exception plus or minus one dilution. It can further be seen from Table 2 that every antiserum reacted with heterologous antigens almost as well as with its homologous antigen. No reactions were obtained with normal rabbit sera.

Cross-absorption tests were then made in which the isolated cell strains were separately compared with HeLa cells. The results are shown in Table 3. It can be seen that the homologous strain absorbed the antibodies as well as the heterologous strains. In some cases an insignificant residue remained which yielded a titre of 1:20.

TABLE 2

RABBIT IMMUNE SERUM COMPLEMENT-FIXATION TITRES * FOR HELA CELLS AND FIVE CELL STRAINS DERIVED FROM HUMAN BREAST CARCINOMA

Rabbit Immune Serum		Cell Strain					
		HeLa	EG	EJ	EV	AR	LR
HeLa	62	320	320	640	640	320	320
	73	160	160	320	320	160	160
	74	320	320	320	320	320	320
EG	34	320	640	640	640	640	640
	71	80	160	160	160	640	640
	72	80	160	160	160	160	640
EJ	33	160	320	320	320	320	320
	67	80	320	320	160	160	160
	68	80	160	160	160	160	160
	76	80	160	160	80	160	80
EV	18	320	320	320	320	640	640
	19	80	80	80	40	160	160
	69	160	320	320	320	320	320
	70	80	160	320	320	160	160
AR	15	160	320	640	320	640	160
	16	40	160	80	80	80	80
	63	320	640	640	640	640	640
	64	160	640	320	320	320	320
LR	35	160	320	320	320	160	160
	65	160	320	320	320	160	160
	66	80	640	160	320	160	320
Normal Rabbit Serum							
	1	0	0	0	0	0	0
	99	0	0	0	0	0	0
	100	0	0	0	0	0	0
	102	0	0	0	0	0	0
	103	0	0	0	0	0	0

* The complement-fixing antibody titres are numerically reciprocals of the serum dilution prior to the addition of the complement, antigen and haemolytic system.

TABLE 3

ABSORPTION AND COMPLEMENT-FIXATION TESTS FOR RABBIT ANTISERA PREPARED AGAINST HELA CELLS AND FIVE DIFFERENT CELL STRAINS DERIVED FROM HUMAN BREAST CARCINOMA

Titres are expressed as reciprocals of the serum dilution as in Table 2

Rabbit Immune Serum	Absorbed with Strain	Titrated with Strain					
		HeLa	EG	EJ	EV	AR	LR
HeLa 62		320	160	320	640	640	320
	HeLa	20	0	0	0	20	20
	EG	20	20				
	EJ	0		20			
	EV	0			0		
	AR	20				40	
	LR	20					20
EG 34		320	640				
	HeLa	0	0				
	EG	20	20				
EJ 33		640		320			
	HeLa	20		0			
	EJ	0		0			
EV 18		160			640		
	HeLa	0			0		
	EV	0			20		
AR 64		640				640	
	HeLa	20				20	
	AR	0				20	
LR 35		80					160
	HeLa	0					0
	LR	0					0

DISCUSSION

Demonstration of complement-fixation for antisera prepared with human cells maintained in tissue cultures has not apparently been reported previously. The complement-fixation observed in the present study for rabbit antisera and HeLa cells and five strains derived from human breast carcinoma is obviously due to antibody formation following immunisation with these cell strains. The antibodies were absorbed not only by the homologous strains but also by the heterologous strains. This indicates the similarity of antigenic structures of the HeLa strain and the five tumour strains isolated by the authors.

SUMMARY

1. Rabbit antisera have been prepared using HeLa cells and five cell strains derived from human breast carcinoma.
2. Complement-fixation tests were carried out using the antisera and the original strains. Closely conforming titres were recorded both for the homologous and for the heterologous strains.
3. The cross-absorption tests revealed that the antibodies are removed by both homologous and heterologous strains.

Acknowledgement. — This investigation was aided by a grant from the Sigrid Juselius Foundation.

REFERENCES

1. Eagle, H.: Proc. Soc. Exper. Biol. & Med. 1955:*89*:362.
2. Gey G. O., Coffman, W. D. and Kubicek, M. T.: Cancer Research: 1952:*12*:264.
3. Horn, E. C.: Ibid. 1956:*16*:595.
4. Miller, D. G. and Hsu, T. C.: Ibid. 1956:*16*:306.
5. Mountain, I. M.: J. Immunol. 1955:*75*:478.
6. Orr, F. and McSwain, B.: Am. J. Path. 1955:*31*:125.
7. Syverton, J. F. Scherer, W. F., and Elwood, P. M.: J. Lab. & Clin. Med. 1954:*43*:286.
8. Winkle, S.: Mikrobiologische und Serologische Diagnostik, Second Edition. Gustav Fischer Verlag, Stuttgart, 1955.